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Low Pulse Oximeter-Measured Hemoglobin Oxygen Saturations With Hemoglobin Cheverly

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Unexpectedly low hemoglobin oxygen saturation as determined by pulse-oximeter analysis was observed in a patient who underwent an elective surgical procedure. Specific hemoglobin derivatives such as carboxyhemoglobin, methemoglobin, and reduced hemoglobin that have been described to lower pulse-oximetry determination of oxygenation were not detected. Absorbance spectra revealed the patient's hemoglobin to be different than that obtained from two normal volunteers. High-pressure liquid chromatographic analysis of the hemoglobin showed an unknown band that comprised 15% of the patient's hemoglobin. DNA sequence analysis showed a point mutation in the second nucleotide of the 45th codon of the β-globin chain. This mutation encodes for an abnormal β-chain (β-45 Phe→Ser) that has been described as hemoglobin Cheverly. Hemoglobin Cheverly is an unstable hemoglobin that has a similar mutation as the β-42 Phe→Ser mutation seen in hemoglobin Hammersmith. Hemoglobin Hammersmith and another unstable hemoglobin, hemoglobin Köln, have previously been described to have unexpectedly low pulse-oximeter–determined oxyhemoglobin levels. That we find hemoglobin Cheverly to result in a similar phenomenon suggests that pulse-oximeter monitoring of oxygenation status may not be appropriate for the unstable hemoglobins. Low pulse-oximeter oxygenation determinations for these hemoglobins do not appear to predict clinical hypoxemia. Am. J. Hematol. 59:181–184, 1998. © 1998 Wiley-Liss, Inc.

Key words: hemoglobin Cheverly; pulse oximeter; oxygen saturation; co-oximetry

INTRODUCTION

Pulse oximetry is an accepted noninvasive method that enables continuous measurement of hemoglobin oxygen saturation [1–3]. The ability of this technique to accurately measure hemoglobin oxygen saturation is dependent upon both instrumental and biological considerations [1–6]. Because these instruments estimate hemoglobin oxygen saturation by measuring light absorption of perfused tissue at 660 and 940 nm during arterial pulses, there are theoretical concerns that differences of hemoglobin absorptive spectra may result in significant misleading estimates in estimated hemoglobin oxygen saturations. For example, increased carboxyhemoglobin and methemoglobin levels result in an overestimation of the pulse-oximeter–determined hemoglobin oxygen saturation as compared with direct arterial co-oximeter analysis [7,8]. Conversely, artifactually low pulse-oximeter–estimated hemoglobin oxygen saturations have been observed in two patients who carried the unstable hemoglobin Köln variant [9,10] and in one patient with the hemoglobin Hammersmith variant [11]. In this report we describe a patient with artifactually low pulse-oximeter–estimated oxygen saturation that is a consequence of the unstable hemoglobin variant, hemoglobin Cheverly.

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TABLE I. Pulse Oximeter Readings and Blood Gas Values

Time	FIO_2	Pulse oximeter O_2 saturation	Blood oximeter PaO_2
Anesthesia induction	30%	86%	
	98%	87%	503 mmHg[a]
After surgery	Air	70–83%	82 mmHg

[a]pH, 7.5; $PaCO_2$, 24; base deficit, 1.7.

CASE REPORT AND RESULTS

A 40-year-old woman with menometrorrhagia for six years and uterine leiomyoma was evaluated for abdominal hysterectomy. Her past medical history was remarkable only for Bell's palsy and asthma for which she occasionally used corticosteroids and inhaled bronchodilators. A tubal ligation had been performed six years previously without incident. She took oral iron intermittently and had a hemoglobin (Hgb) of 12.0 g/dl three years previously. Physical examination was remarkable only for pallor, mild obesity, a soft systolic murmur at the base of the heart, and a mass in the lower abdomen extending 11 cm above the symphysis pubis. Pelvic examination confirmed a large uterus the size of a three- to four-month pregnancy due to the leiomyomas. No clubbing was present. Retrospectively, some observers believed that she appeared slightly cyanotic.

A complete blood count four days prior to admission revealed white blood cell (WBC) count 7.1×10^9/L, leukocyte differential count 30% lymphocytes, 67% neutrophils, 3% monocytes, Hgb 11.4 g/dL, hematocrit (Hct) 35.4%, mean corpuscular volume (MCV) 83.3 fL, corpuscular hemoglobin concentration (MCHC) 32.2 g/dL, red cell distribution width (RDW) 13.6, platelet count 374×10^9/L. A chemistry profile, that included a bilirubin (total and direct) and lactate dehydrogenase (LDH), was normal. There was no laboratory evidence for hemolysis. An electrocardiogram showed nonspecific inferior wall T-wave abnormalities, and possible anteroseptal infarct, age indeterminate. Serum folic acid, vitamin B_{12} and ferritin, and a chest x-ray were normal.

Anesthetic premedication included oral omeprazole, metoclopramide, and prednisone followed by intramuscular meperidine, hydroxyzine, and atropine. A pulse oximeter (Nellcor model N-2500, Pleasanton, CA) was attached just as the anesthetic induction started. It revealed an oxyhemoglobin saturation of 86% on an inspired atmosphere of 30% oxygen and 70% nitrous oxide. Shown in Table I are these values as well as the results of blood oximetry readings obtained with an Instrumental Laboratory model 482 (Lexington, MA) co-oximeter at both 30 and 98% FIO_2. Identical blood oximetry readings were obtained with an Instrumental Laboratory model IL130G blood-gas analyzer.

The co-oximeter values for specific Hgbs showed only

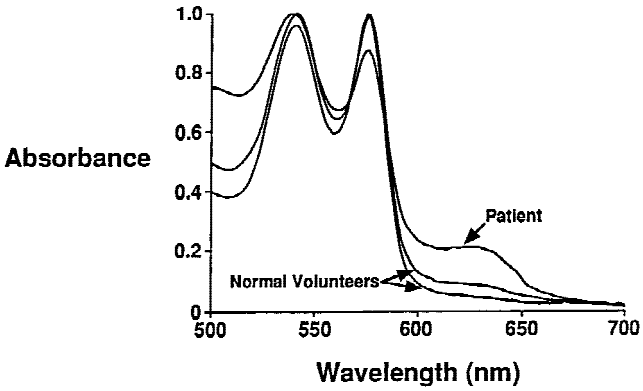

Fig. 1. Absorbance spectra of hemoglobin from two normal volunteers superimposed on the spectra from the patient. The patient's spectral curve begins at 0.75 absorbance.

2.2% carboxyhemoglobin, 3.7% methemoglobin, and 3.4% reduced hemoglobin. With these results revealing adequate delivery of oxygen to the blood with only slight elevation of carboxyhemoglobin (reference range <1.5) and methemoglobin (reference range 0.4–1.5) and with continuing clinical stability of the patient, it was elected to proceed with surgery. Hysterectomy was accomplished, the patient had an uneventful postoperative course, and was discharged on the third postoperative day.

Oxygen saturation measurements were made with other pulse oximeter probes both in the operating room and the recovery room, and during the next few days, all with consistently low saturations (Table I). Pulse oximeter probes placed on earlobes, nose, digits, and extremities were similarly low. Potential confounding factors for pulse oximetry such as movement artifacts and ambient light artifacts were excluded. Further questioning revealed that she lives in town, drinks city water, is not aware of any exposure to chemicals, and has no unusual hobbies. Postoperative pulmonary function tests were entirely normal. An echocardiogram with bubble study did not reveal an occult right-to-left shunt.

Postoperative peripheral venous blood, anticoagulated in ethylenediaminetetraacetic acid (EDTA), was obtained. Red blood cells (RBCs) were fractionated from plasma by centrifugation at 1,000 g for ten min and subsequently washed three times with 0.162 N NaCl. Packed RBCs were lysed by rapid freeze–thawing times three in a dry ice/acetone bath. Hemolyzate was diluted with 0.162 N NaCl prior to spectrophotometric analysis on a Hewlett Packard 8452A Diode Array Spectrophotometer (Palo Alto, CA). The absorbance spectrum of the patient's hemoglobin was compared with comparably diluted hemoglobin obtained from two normal volunteers. These spectra are shown in Figure 1. These spectra demonstrate that at 660 nm, the wavelength at which pulse

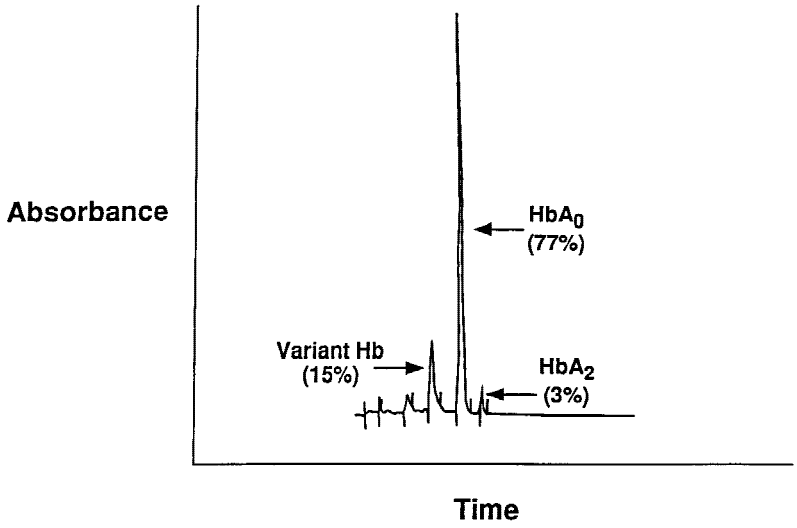

Fig. 2. HPLC chromatogram demonstrating the unknown variant. Absorbance was measured at 415 nm.

oximeter readings are obtained, there are differences in the absorbance between hemoglobin Cheverly and hemoglobin obtained from normal volunteers.

Hemoglobin from the patient was also subjected to cellulose acetate, citrate agar, and high pressure liquid chromatographic (HPLC) analysis. The cellulose acetate and citrate agar electrophoretic gels both displayed normal banding patterns but an abnormal hemoglobin was detected in an HPLC chromatogram using a Poly CAT A cationic ion exchange column (Poly LC, Columbia, MD) with an acetate gradient in 0.3 M bis Tris buffer [12]. Hemoglobin quantitation revealed that the unknown hemoglobin variant comprised 15% of the patient's hemoglobin (Fig. 2). Sequence analysis of the amplified β-globin gene [13] detected a T→C mutation in the second nucleotide of the 45th codon of the β-globin gene (data not shown). This point mutation results in a phenylalanine to serine substitution in amino acid 45 in the β-globin chain. This variant has previously been described and named hemoglobin Cheverly [14,15].

DISCUSSION

There are two reports in the literature that describe the β-globin chain variant that has been named hemoglobin Cheverly [14,15]. The abnormality was discovered in a seven-year-old child who was evaluated for mild chronic anemia [15]. As with the patient described in this present report, electrophoresis of blood on both cellulose acetate and citrate agar revealed normal patterns. Functional studies that were obtained on the red cells from the initial proband demonstrated that hemoglobin Cheverly has a reduced affinity for oxygen and reduced Bohr effect [15]. The amino acid substitution in hemoglobin Cheverly (β-45 Phe→Ser) is similar to that in hemoglobin Hammersmith (β-42 Phe→Ser) [14,15]. However, the mutation in hemoglobin Hammersmith results in a more severe hemoglobin instability than does the mutation causing hemoglobin Cheverly, likely because of a more dramatic alteration of the conformation of the heme pocket seen with mutations of β-42 [16].

Our present report demonstrates that hemoglobin Cheverly can cause an artifactually low pulse-oximeter–estimated oxygen saturation as compared to the blood co-oximeter analysis. This is an important finding because most hemoglobinopathies that are dysfunctional result in overestimations of the pulse-oximeter–measured oxygen saturation. Pulse oximeters measure oxyhemoglobin as a percentage of functional hemoglobin (oxyhemoglobin + deoxyhemoglobin) whereas blood co-oximeters that are used for formal blood gas analyses measure oxyhemoglobin as a measure of total hemoglobin (oxyhemoglobin + deoxyhemoglobin + carboxyhemoglobin + methemoglobin + other hemoglobins) [7,8]. Thus, increased levels of a dysfunctional hemoglobin, such as methemoglobin, would result in an accurate blood co-oximeter measurement of oxyhemoglobin but an incorrect high pulse-oximeter value. The pulse-oximeter readings were also carefully performed to eliminate other potential confounding factors for accurate readings such as movement artifact, tissue hypoperfusion, and ambient light artifacts [3].

The unstable hemoglobins Köln [9,10] and Hammersmith [11] have previously been described to produce a low estimated pulse-oximeter oxyhemoglobin percentage, as we have observed in this current patient with hemoglobin Cheverly. The reason for this artifactually low reading for hemoglobin Köln is not known, and, in fact, is unexpected because hemoglobin Köln has an abnormally high oxygen affinity [17,18] and would there-

fore be expected to have a high pulse-oximeter–determined oxyhemoglobin level while the patient is breathing room air. Hemoglobin Hammersmith, because of its instability, is virtually all in an oxidized form which has an absorption spectrum that is isobestic with deoxyhemoglobin at 660 nm [11]. In contrast, hemoglobin Cheverly has a decreased oxygen affinity [14] and one might therefore anticipate that patients with hemoglobin Cheverly would have diminished oxyhemoglobin levels. The patient described in this present report had marked reductions in oxyhemoglobin levels when determined using the pulse oximeter, and these were much lower than would be predicted from the co-oximetry readings shown in Table I. Furthermore, shifts of the oxyhemoglobin dissociation curve alone should not alter the pulse-oximeter–determined oxyhemoglobin levels unless a hemoglobin variant with different spectral properties at 660 and 940 nm is also present [3–7]. That we find hemoglobin Cheverly to also result in an artifactually low pulse-oximeter oxyhemoglobin level as did hemoglobins Köln [9,10] and Hammersmith [11], raises the possibility that this pulse oximetry abnormality is a general property of unstable hemoglobins.

Pulse oximetry may therefore be an inaccurate method in which to follow patients with some types of unstable hemoglobins. This contrasts with the accepted use of pulse oximetry for noninvasively monitoring patients with other hemoglobinopathies, such as sickle cell disease, where reasonably detailed studies have validated its usefulness to accurately assess hypoxemia [19,20]. Because of the dependence of pulse oximetry on hemoglobin absorptive spectra and the likelihood that amino-acid substitutions, particularly in the heme oxygen binding pocket, may alter the absorptive spectra, then hemoglobin analyses to assess for the presence of unstable hemoglobins should be an important part of the evaluation of a patient with an unexplained discordance in pulse oximetry and blood co-oximetry. Furthermore, cellulose acetate and citrate agar electrophoretic analysis of the hemoglobin may be insufficient to assess for variants, such as hemoglobin Cheverly, which co-migrates with hemoglobin A.

Hemoglobins with a low affinity for oxygen may be associated with mild cyanosis and a slightly low hemoglobin level, but are usually asymptomatic and require no special management [21]. Our observations demonstrating abnormal readings by pulse oximeter should be recognized to avoid inappropriate responses at the time of anesthesia or monitoring of oxygenation status otherwise.

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REFERENCES

1. Severinghaus JW, Astrup PB: History of blood gas analysis. IV. Oximetry. J Clin Monit 2:270, 1986.
2. Severinghaus JW, Honda Y: History of blood gas analysis. VII. Pulse oximetry. J Clin Monit 3:135, 1987.
3. Bowes WA, Corke BC, Hulka J: Pulse oximetry: A review of the theory, accuracy, and clinical applications. Obstet Gynecol 74:541, 1989.
4. Yelderman M, New W: Evaluation of pulse oximetry. Anesthesiology 59:349, 1983.
5. Chapman KR, Liu FLW, Watson RM, Rebuck AS: Range of accuracy of two wavelength oximetry. Chest 89:540, 1986.
6. Cecil WT, Thorpe KJ, Fibuch EE, Tuohy GF: A clinical evaluation of the accuracy of the Nellcor N-100 and Ohmeda 3700 pulse oximeters. J Clin Monit 4:31, 1988.
7. Barker SJ, Tremper KK: The effect of carbon monoxide inhalation on pulse oximetry and transcutaneous PO_2. Anesthesiology 66:677, 1987.
8. Barker SJ, Tremper KK, Hyatt J: Effects of methemoglobinemia on pulse oximetry and mixed venous oximetry. Anesthesiology 70:112, 1989.
9. Katoh R, Miyake T, Arai T: Unexpectedly low pulse oximeter readings in a boy with unstable hemoglobin Köln. Anesthesiology 80:472, 1994.
10. Gottshalk A, Silverberg M: An unexpected finding with pulse oximetry in a patient with hemoglobin Köln. Anesthesiology 80:474, 1994.
11. Lang SA, Chang PC, Laxdal VA, Huisman: Haemoglobin Hammersmith precludes monitoring with conventional pulse oximetry. Can J Anaesth 41:965, 1994.
12. Wilson JB, Huisman THJ: Detection and quantitation of normal and abnormal hemoglobins in adults and newborns by HPLC. In Huisman THJ (ed): The Hemoglobinopathies. New York: Churchill Livingston, 1986, p 32.
13. Divoky V, Bisse E, Wilson JB, Gu L-H, Wieland H, Heinrichs I, Prior JF, Huisman THJ: Heterozygosity for the IVS-I-5 (G→C) mutation with a G→A change at codon 18 (Val→Met; Hb Baden) in cis and a T→G mutation in trans resulting in thalassemia intermedia. Biochim Biophys Acta 1180:173, 1992.
14. Sciarrata GV, Sansone G, Valbonesi M, Wilson JB, Lam H, Webber BB, Headlee ME, Huisman THJ: Hb Cheverly or $\alpha_2\beta_2$ 45 (CD4) Phe→Ser in an elderly Italian male. Hemoglobin 6:419, 1982.
15. Yeager AM, Zinkham WH, Jue DL, Winslow RM, Johnson MH, McGuffey JE, Moo-Penn WF: Hemoglobin Cheverly: β45 (CD4) Phe→Ser, an unstable hemoglobin associated with chronic mild anemia. Pediatr Res 17:503, 1984.
16. Dacie JV, Shinton NK, Gaffney PJ, Carrell RW, Lehmann H: Hemoglobin Hammersmith (β42(CD1)Phe→Ser). Nature 216:663, 1967.
17. Woodson RD, Heywood JD, Lenfant C: Oxygen transport in hemoglobin Köln: Effect of increased oxygen affinity in absence of compensatory erythrocytosis. Arch Intern Med 134:711, 1974.
18. de Furia FG, Miller DR: Oxygen affinity in hemoglobin Köhn disease. Blood 43:527, 1972.
19. Weston Smith SG, Glass UH, Acharya FJ, Pearson TC: Pulse oximetry in sickle cell disease. Clin Lab Haemat 11:185, 1989.
20. Rackoff WR, Kunkel N, Silber JH, Asakura T, Ohene-Frempong K: Pulse oximetry and factors associated with hemoglobin oxygen desaturation in children with sickle cell disease. Blood 81:3422, 1993.
21. Stamatoyannopoulos G, Parer JT, Finch CA: Implications of a hemoglobin with decreased oxygen affinity (hemoglobin Seattle) N Engl J Med 281:915, 1969.